Zinc isotope fractionation during grain filling of wheat and a comparison of Zn and Cd isotope ratios in identical soil-plant systems

Matthias Wiggenhauser[1], Moritz Bigalke[2], Martin Imseng[2], Armin Keller[3], Corey Archer[4], Wolfgang Wilcke[5], Emmanuel Frossard[1]

[1]Institute of Agricultural Sciences, ETH Zürich, Eschikon 33, CH-8315 Lindau, Switzerland 8
[2]Institute of Geography, University of Bern, Hallerstr. 12, CH-3012 Bern 9
[3]Swiss Soil Monitoring Network (NABO), Agroscope, Reckenholzstrasse 191, CH-8046 Zürich 11
[4]Department of Earth Science, ETH Zurich, Clausiusstrasse 25, CH-8092 Zürich
[5]Institute of Geography and Geoecology, Karlsruhe Institute of Technology (KIT), P.O. Box 6980, D-76049 Karlsruhe

Author for correspondence: Matthias Wiggenhauser, matthias.wiggenhauser@usys.ethz.ch, Tel: +41 (0) 52 354 91 43

Short title (< 93 character) for New Phyt Twitter account: Zn isotope fractionation during grain filling & Zn and Cd isotope ratios in wheat

total word count (excluding summary, references and legends:)	words	No. of figures (all colour):	No. of tables:	No of SI files:
total	6123	4	2	1
Introduction	1198			
Materials and Methods	1343			
Results	1223	3	2	
Discussion	2294	1		
Acknowledgements	65			

Abstract (200)

- Remobilization of Zn from shoot to grain contributes significantly to Zn grain concentrations and thereby to food quality. On the other hand, strong accumulation of Cd in grain is detrimental for food quality.

- Zn concentrations and isotope ratios were measured in wheat shoots (*Triticum aestivum* L.) at different growth stages to elucidate Zn pathways and processes in the shoot during grain filling.

- Zinc mass significantly decreased while heavy Zn isotopes accumulated in straw during grain filling ($\Delta^{66}Zn_{fullmaturity\text{-}flowering}$ = 0.21 to 0.31 ‰). Three quarters of the Zn mass in the shoot moved to the grains, which were enriched in light Zn isotopes relative to the straw ($\Delta^{66}Zn_{grain\text{-}straw}$ -0.21 to -0.31 ‰).

- Light Zn isotopes accumulated in phloem sinks while heavy isotopes were retained in phloem sources likely because of apoplastic retention and compartmentalization. Unlike for Zn, an accumulation of heavy Cd isotopes in grains has previously been shown. The opposing isotope fractionation of Zn and Cd might be caused by distinct affinities of Zn and Cd to O, N and S ligands. Thus, combined Zn and Cd isotope analysis provides a novel tool to study biochemical processes that separate these elements in plants.

Keywords (5-8)

Zinc, Cadmium, Isotope ratios, Wheat, Remobilization, Element speciation

Introduction

Zinc is an essential micronutrient for all biota. In plants, Zn is a cofactor for more than 300 enzymes (Coleman, 1998). Thus, Zn plays an important role in numerous metabolic processes such as detoxification of oxygen radicals or the maintenance of membrane integrity (Broadley *et al.*, 2012). Consequently, an adequate Zn supply, efficient Zn uptake mechanisms, and the redistribution of Zn into the different plant organs are crucial for regular plant growth (Page & Feller, 2015). In human nutrition, sufficiently high Zn concentrations in edible plant parts are vital to prevent Zn deficiencies (Frossard *et al.*, 2000; Palmgren *et al.*, 2008). Thus, understanding of uptake and remobilization processes of Zn during the grain filling period of cereals is essential (Pottier *et al.*, 2014; Yamaji & Ma, 2014).

Once Zn is taken up by the roots, a complex long-distance transport system facilitates the distribution of Zn to the vegetative and reproductive organs. In the roots, Zn is loaded into the xylem, which is directed to transpiring organs such as expanded leaves. The osmotically driven phloem directs Zn to organs that are not in the main xylem stream such as young leaves, emerging roots, and grains (Yamaji & Ma, 2014). During grain filling, nutrients in senescent leaves and stems are remobilized from the straw and transported via the phloem into the grains (Pottier *et al.*, 2014). In addition, during grain filling, the plant is still taking up Zn from the soil, which is continuously loaded into the xylem, directly transferred into the phloem, and transported into the grains without an intermediate storage in the leaves and stems (Yoneyama *et al.*, 2015). For both pathways, Zn needs to cross several apoplastic barriers (Olsen & Palmgren, 2014) and changes its chemical environment several times (Yoneyama *et al.*, 2015).

Many metal chelators and membrane proteins are involved in Zn transport from soil to grain (Yamaji & Ma, 2014; Alvarez-Fernandez *et al.*, 2014). Unfortunately, the substrate specificity of these transporters is limited (Khan *et al.*, 2014), which leads to an unintended uptake of the toxic and non-essential

element Cd. Because of the accumulative characteristics of Cd in human bodies, its small but continuous intake can harm organs such as the kidneys (Godt *et al.*, 2006). Therefore, the strongly linked uptake and redistribution processes of these elements need to be understood in order to foster and reduce Zn and Cd concentrations in edible plant parts, respectively,

In the past decade, various studies have analyzed Zn isotope ratios in several plant species under varying Zn supply (reviewed in Caldelas & Weiss, 2017). The plants showed distinguishable Zn isotope fractionation patterns that were caused by distinct biogeochemical processes. For instance, heavier isotopes preferentially fractionate into shorter (or harder) bonds and lighter isotopes into longer (or softer) bonds during equilibrium fractionation, while incomplete chemical reactions or physical transport processes such as adsorption, membrane transport, change of metal speciation, cellular compartmentalization and diffusion result in kinetic isotope fractionation (Fry, 2006; Balter *et al.*, 2013; Caldelas & Weiss, 2017). Therefore, Zn isotope fractionation in plants contains information about the main processes that determine uptake and distribution of Zn in plants. The integration of Zn isotope data into existing conceptual models of Zn uptake and transport in plants can provide additional indications of the main mechanisms that determine Zn concentrations in grains.

To date, most studies focused on Zn isotope fractionation during plant uptake and less data are available on Zn isotopic fractionation in plant shoots (Caldelas & Weiss, 2017). Leaves of trees in a pristine tropical watershed were enriched in light isotopes compared to the stems, which was attributed to a continuous removal of heavy Zn isotopes into the adjacent cells of the xylem vessels (Viers *et al.*, 2007). In contrast, combined Zn isotope and speciation measurements in a reed canary grass revealed that light Zn isotopes accumulated in stems and heavy Zn isotopes in leaves (Aucour *et al.*, 2015). The Zn isotope fractionation within the shoot has been explained by Zn remobilization from leaves via the phloem to stems where the majority of the Zn was stored. Moreover, the enrichment of heavy Zn isotopes in leaves has been ascribed to sorption to cell walls, xylem unloading, and storage in vacuoles by binding Zn to organic acids

(Aucour *et al.*, 2015). Moynier et al. (2009) observed a positive correlation between the enrichment of light Zn isotopes and the height of leaves above the soil in bamboo. A similar relation has been found in reed, where the highest and youngest leaves were isotopically lighter than the lower and mature leaves (Caldelas *et al.*, 2011). In summary, Zn isotope fractionation between stem and leaves varies between plant species which might be related to age and height of plant tissues but also to the Zn distribution within the plant shoot. To date, only one study measured Zn isotope ratios in reproductive organs at full maturity. Arnold et al. (2015) have shown that grains of rice were significantly enriched in light isotopes compared to the remaining shoot. The preferential storage of the light Zn isotopes in the grains has been explained by xylem-phloem transfer and the entry and reentry of the symplastic space during the transport into the grain. To date, no study has measured Zn isotope ratios in different shoot organs at different growth stages. Such data could provide information about pathways and processes of Zn that control the transfer of Zn into the grain.

A recently published study has shown that wheat grains are enriched in heavy Cd isotopes compared to straw and root (Wiggenhauser *et al.*, 2016). The opposing isotope fractionation of Cd in wheat and Zn in rice (Arnold *et al.*, 2015) suggests that isotope ratios of these metals could be used to study the distinct redistribution processes of chemically similar nutrients and pollutants. We are not aware of a direct comparison of Zn and Cd isotope fractionation in identical soil-plant systems.

In this study, we focused on the redistribution of Zn isotopes in wheat shoots during the grain-filling period. For that purpose, we sampled shoots grown on arable soils under controlled conditions. Zinc isotopes were measured in various shoot organs at the onset and end of grain filling. In order to compare Zn with Cd isotope fractionation, we used the same wheat cultivar and soils as in a previous study about Cd isotope fractionation (Wiggenhauser *et al.*, 2016). The objectives of the study were to (a) determine and compare the Zn isotope fractionation among various organs of the wheat shoot at flowering and at full maturity. Furthermore, we aimed to (b) elucidate pathways of Zn isotopes in the

shoot and to refine existing conceptual models that describe biochemical process that cause Zn isotope fractionation within plant shoots (c) and compare the isotope fractionation of Zn and Cd in identical soil-wheat systems. We expected that Zn isotope ratios become significantly fractionated between flowering and grain filling since Zn is remobilized from different organs in the shoots towards the grains, which involves several processes that are prone to fractionate Zn isotopes. Furthermore, we expected that Zn and Cd isotope fractionation differs in identical soil-plant systems because of distinct biochemical processes in plants that discriminate these chemically similar elements.

Materials and Methods

Plant growth and sampling

The wheat cultivar (*Triticum aestivum* L., cv. "Fiorina") and arable soils from the Swiss Soil Monitoring Network (Gubler et al. 2015) from Oensingen (Oen) and Landquart (LQ) were representative for Swiss wheat production (Table S1). The soils had similar Zn concentrations (LQ 96 ± 7.7 mg kg^{-1}, Oen 100 ± 1.0 mg kg^{-1}, Table S1) but differed in pH (LQ 7.1, Oen 5.6). The latter was expected to drive the plant availability of Zn in these soils (Mertens & Smolders, 2013). The chosen soil-wheat systems were identical with the ones previously used to study Cd isotope fractionation in soil-wheat systems (Wiggenhauser *et al.*, 2016).

Wheat was grown in pots that contained 1 kg of dry soil. The wheat plants were cultivated in a growth chamber with the following settings: photoperiod 16 h:8 h, light:dark, illuminance 25 klx, temperature 24°C at daytime and 16°C at night, humidity 60%. To ensure regular plant growth, 100 mg N (80 mg N applied as $Ca(NO_3)_2$ and 20 mg as NH_4NO_3), 35 mg P (KH_2PO_4), 50 mg K (K_2SO_4), 25 mg Mg ($MgSO_4$), and 5 mg Fe (Fe-EDTA) were added to each pot and mixed thoroughly. The soils were watered to maintain a range of 40 to 70%

of the maximal water holding capacity (Table S1). For each soil, eight pots were prepared. The wheat was sampled at flowering and at full maturity. The time periods before flowering and between flowering and grain filling are considered as vegetative growth and grain filling periods, respectively. Four out of eight replicates were sampled at flowering and four at full maturity. At flowering, the wheat was sampled as soon as tillers number 1-3 were flowering and the total number of tillers was recorded. Tillers 1-3 were dissected into flag leaves, peduncle, rachis, spikelets, lower shoots, and senescent leaves (illustrated in Fig. S1). The remaining shoot biomass was defined as lower straw. At full maturity, flag leaves, peduncle, rachis, spikelets, and grains were sampled for all tillers (Fig. S1). The remaining shoot biomass was also defined as lower straw. After sampling, shoot organs were rinsed with >18.2 MΩ H_2O. At full maturity, roots were also sampled to compare isotope fractionation of Zn and Cd (Wiggenhauser et al., 2016) in the same soil-plant systems. The roots were cleaned using 6 mM $NaNO_3$ as described previously (Wiggenhauser *et al.*, 2016).

Sample preparation

Plant and soil samples were ground in a rotary mill equipped with tungsten carbide cups. Plant material (0.2 g) was digested in a high-pressure single reaction chamber microwave system (turboWave, MWS microwave systems) using 4 mL distilled 6 M HNO_3. Duplicates of the initially sampled soils were digested on the hotplate (130°C) in closed teflon beakers using a mixture of 2 mL HNO_3 and 2 mL HF. Additionally, 0.1 M HCl extractions of the same soils were prepared to estimate plant available Zn (Ponnamperuma *et al.*, 1981) as previously used to study Zn isotope fractionation in soil-plant systems (Arnold *et al.*, 2010, 2015; Smolders *et al.*, 2013).

Sample purification and isotope analysis

Aliquots of the digested samples were taken to determine Zn concentrations with quadrupole ICP-MS. Additionally, concentrations of micro- and macronutrients as well as Cd were also determined using ICP-MS (Cd, Cu, Co,

Mn, Ni, and Fe), ICP-OES (Ca, K, Mg, and P), and Element Analyzer (C, N, and S; details in Table S2). Three out of the four individually digested plants were further purified for isotope analysis using an adapted protocol from Pinna et al. (2001) and Bermin et al. (2006). Polypropylene columns (Spectrum Laboratory, Spectra/Chrom® Minicolumns) were filled with an anion-exchange resin (2 mL, Bio-Rad laboratories, AG® 1-X8, 100-200 mesh). After cleaning of the resin with 2 M HNO_3 (10 mL) and 0.5 M HCl (5 mL), the columns were conditioned with 6 M HCl (10 mL). The samples were loaded in 6 M HCl (5 mL) and the sample matrix was eluted adding consecutively 6 M HCl (10 mL), 2.5 M HCl (5 mL) and 0.5 M HCl (15 mL). Finally, the samples were eluted using 0.3 M HNO_3 (10 mL), evaporated to dryness and resolubilized in 0.3 M HNO_3 (5 mL). Afterwards, aliquots of the samples containing 100 ng of Zn were taken and mixed with 100 ng of the double spike consisting of the ^{67}Zn and ^{64}Zn isotopes (Bermin *et al.*, 2006). The blank samples were evaporated to dryness and amended with 50 ng of the $^{67/64}Zn$ double spike. The mixture was then evaporated to dryness and resolubilized in 0.3 M HNO_3 (1 mL). For each digestion and purification batch, a soil or plant standard reference material and a blank were processed together with the samples. All acids used were double distilled to keep the blank as low as possible. The contribution of the procedural blanks were low and ranged from 0.3 to 3.8 % to the total Zn mass of the samples and their impact on the Zn isotope ratios was therefore expected to be negligible (Table S3a). Measurements of the different standard reference materials revealed high average Zn recoveries (96 to 103 %, Table S3b).

Isotope ratios were measured using a Thermo Scientific Neptune Plus high-resolution multicollector ICP-MS following the protocol of Bermin et al. (2006) and Zhao et al. (2014). Samples were introduced with an Aridus desolvating nebulizer system (Cetac, Omaha, NE, USA). The $^{67/64}Zn$ double spike was used to correct for mass bias drifts during the measurements and to calculate Zn concentrations of the samples using isotope dilution (Archer & Vance, 2004; Bermin *et al.*, 2006). The Zn isotope data acquired with SRMs revealed good reproducibilities of 2sd between 0.02 and 0.05 ‰ of n = 2 to 6 samples and

isotope ratios agreed well with ratios obtained in other laboratories (Table S3b). Long-term reproducibility of Zn isotopic analyses was ± 0.06 ‰. This was assessed over the course of this and parallel studies, through repeated measurements of a primary (JMC Lyon) and a secondary Zn isotope standard (IRMM-3702). These latter give $^{66}Zn_{JMC\text{-}Lyon}$ = +0.30 ± 0.06 (2 sd, n = 350 at the time of writing (Moeller *et al.*, 2012; Archer *et al.*, 2017).

Calculations and statistics

The isotope compositions of the samples are reported relative to JMC Lyon Zn isotope standard using a δ notation based on the $^{66}Zn/^{64}Zn$ ratio:

$$\delta^{66}Zn = \left[\frac{\left(^{66}Zn/^{64}Zn\right)_{sample}}{\left(^{66}Zn/^{64}Zn\right)_{LyonJMC}} - 1 \right] \times 1000 \quad \text{(Eqn. 1)}$$

The apparent isotopic fractionation during grain filling was calculated using the Δ notation:

$$\Delta^{66}Zn_{grain.filling} = \delta^{66}Zn_{fullmat} - \delta^{66}Zn_{flowering} \quad \text{(Eqn. 2)}$$

where "fullmat" and "flowering" denote the time of sampling (full maturity, flowering) and "grain.filling" the period between flowering and full maturity. The same equation was also used to calculate the apparent isotope fractionation between different compartments of the soil-plant system (e.g., $\Delta^{66}Zn_{wheat\text{-}exctract}$)

The isotope ratios of plant organs that consist of several individually measured organs such as e.g., straw, shoot or whole plant were determined using weighted mean calculations with the Zn mass as weighting factor as described previously (Wiggenhauser *et al.*, 2016). Mass balances for each individual wheat organ were calculated for a single tiller (i.e. the Zn masses of an individual organ were divided by the number of sampled tillers).

To determine if differences in Zn mass and Zn isotope ratios among the wheat organs (n ≥ 3 biological replicates) were statistically significant within the same growth period, we used ANOVA followed by a Tukey HSD test if a significant

effect was detected. The changes of Zn masses and isotope ratios during grain filling were calculated by subtracting mean Zn masses and isotope ratios at flowering from mean masses and isotope ratios at full maturity:

$$mean_{grain.filling} = mean_{fullmat} - mean_{flowering} \quad \text{(Eqn. 3)}$$

The standard error of these mean values were calculated using an error propagation:

$$SE_{grain.filling} = \sqrt{(SE_{fullmat})^2 + (SE_{flowering})^2} \quad \text{(Eqn. 4)}$$

where SE denotes standard errors of Zn mass and Zn isotope ratios measured at full maturity or at flowering. Significant differences of these mean values were then tested using t-tests.

For all statistical tests and correlations between variables (Pearson's correlation coefficient), the level of significance was set to $p < 0.05$. Statistical tests, correlations and plots were computed using the statistical software R (version 3.1.3).

Results

Soil properties and plant growth

The 0.1 M HCl-extractable Zn concentration, considered as plant-available Zn, was significantly lower in LQ (0.40 ± 0.01 mg kg^{-1}) than in Oen (10.9 ± 0.11 mg kg^{-1}), likely because of the higher soil pH in LQ compared with Oen (Mertens & Smolders, 2013).

At flowering, total shoot dry weight (DW) were 5.61 ± 0.25 g and 6.42 ± 0.91 g for LQ and Oen, respectively (Table 1). Among the shoot organs, the lower straw accounted for 62 to 71 % of the total DW of the shoots while the spikelets accounted for 15 to 18 % of the total shoot DW. All other shoot organs accounted for less than 6 % of the total shoot DW. At full maturity, the grain

accounted for 47 to 54 % of the total DW of the wheat shoot, lower straw for 21 to 31 %, spikelets for 15 to 17 %, and the remaining organs for less than 4 %. During grain filling, total shoot DW increased by a factor of 2 to 3 in Oen and LQ, respectively. Moreover, the straw DW increased by a factor of 1.1 and 1.5 in Oen and LQ, respectively.

Zinc concentrations and mass in wheat shoots

Total Zn concentrations in shoots at flowering were 28.5 ± 1.2 mg kg^{-1} in LQ and 15.8 ± 1.1 mg kg^{-1} in Oen (Table 1). Moreover, lower shoot Zn concentrations in Oen than in LQ were unexpected, because the 0.1 M HCl extracted about 25 times more Zn from the Oen soil than from the LQ soil (Table S2). A detailed discussion of disagreement of the Zn concentrations in the 0.1 M HCl extract and the wheat shoots is given in Notes S1.

At flowering, the Zn concentrations in the straw organs ranged in LQ from 18.0 to 29.5 mg kg^{-1} and in Oen from 7.4 to 22.3 mg kg^{-1} (Table 1). In LQ, the Zn concentrations in the flag leaves, spikelets, and lower straw (26.9 to 29.5 mg kg^{-1}) were significantly higher than in rachis, peduncle, and senescent leaves (18.0 to 21.5 mg kg^{-1}). In Oen, the Zn concentrations in the spikelets, rachis, and flag leaves were significantly higher (17.2 to 22.3 mg kg^{-1}) compared with the senescent leaves, lower straw, and peduncle (7.4 to 15.2 mg kg^{-1}). At full maturity, the Zn concentrations were highest in the grains (25.8 to 33.3 mg kg^{-1}) and ranged in the straw organs from 5.9 to 18.0 mg kg^{-1} in LQ and from 4.7 to 14.6 mg kg^{-1} in Oen.

During grain filling, the shoot took up additional Zn from the soil (15.6 to 19.7 µg per tiller, Table S4). Furthermore, the grains accumulated more Zn (25.8 to 33.3 µg per tiller) than the shoot accumulated additionally within this period. These calculations show that part of the Zn in the grain derived from the Zn pool in the straw that accumulated during vegetative growth and that was later remobilized during grain filling. Fig. 1 illustrates that the different shoot organs lost distinct quantities of Zn mass. In LQ, lower straw lost most Zn mass during grain filling (-7.25 ± 0.73 µg), followed by spikelets and flag leaf (-3.52 to

-2.79 µg) and by rachis and peduncle (-0.20 to 0.15 µg). In Oen, spikelets and lower straw lost most Zn mass during grain filling (-5.70 to -2.76 µg), followed by flag leaf (-1.25 ± 0.22 µg) and peduncle and rachis (-0.39 to -0.05 µg). At full maturity, the Zn in the grains accounted for 76 to 77 % of total Zn mass in the shoot (Table S4).

Zinc isotope ratios in soils and whole wheat

Zinc isotopes were significantly fractionated in both soil-plant systems at full maturity (Fig. S2). Bulk soils of LQ and Oen had indistinguishable Zn isotope ratios (δ^{66}Zn: LQ 0.18 ± 0.01 ‰, Oen 0.16 ± 0.01 ‰). The Zn isotope ratios in the HCl soil extracts were significantly heavier than in the total soil digests and Zn isotope ratios in the HCl extracts were heavier in LQ than in Oen (δ^{66}Zn: LQ 0.60 ± 0.03 ‰, Oen 0.37 ± 0.08 ‰). Relative to the HCl extracts, the whole wheat plant at full maturity was depleted in heavy Zn isotopes in LQ ($\Delta^{66}Zn_{wheat\text{-}extract}$: -0.33 ‰) but enriched in heavy Zn isotopes in Oen ($\Delta^{66}Zn_{wheat\text{-}extract}$: 0.13 ‰). The strong enrichment of heavy isotopes in the wheat grown on Oen further supports the hypothesis that these plants were insufficiently supplied with Zn which is discussed in detail in Notes S2.

Zinc isotope ratios in wheat shoots

The Zn isotope ratios in the whole shoot changed during grain filling (Fig. 2). In LQ, the shoots were significantly enriched in heavy Zn isotopes at full maturity relative to flowering ($\Delta^{66}Zn_{grain.filling}$: 0.07 ± 0.01 ‰). However, a similar enrichment of heavy Zn isotopes during grain filling was not significant in Oen ($\Delta^{66}Zn_{grain.filling}$: 0.05 ± 0.06 ‰).

Zinc isotope ratios differed significantly among the shoot organs at both growth stages (Fig. 3a). At flowering, spikelets were significantly enriched in light Zn isotopes (δ^{66}Zn: LQ -0.13 ± 0.01 ‰, Oen 0.28 ± 0.06 ‰) compared with the other organs for both soils (δ^{66}Zn: LQ 0.05 to 0.46 ‰, Oen 0.52 to 0.72 ‰). The strongest enrichment in heavy isotopes was found in senescent leaves (δ^{66}Zn: LQ 0.46 ± 0.12 ‰, Oen 0.72 ± 0.02 ‰). At full maturity, the grains and

spikelets were enriched in light Zn isotopes in both soils (δ^{66}Zn: LQ 0.18 to 0.36 ‰, Oen 0.45 to 0.47 ‰). The strongest enrichment of heavy isotopes was found in peduncle and rachis (δ^{66}Zn: LQ 0.76 to 0.78 ‰, Oen 0.78 to 0.97 ‰).

Zinc isotopes in the straw organs changed during grain filling in a range of $\Delta^{66}Zn_{grain.filling}$ from 0.14 to 0.62 ‰ (Fig. 3b). The apparent isotope fractionation during grain filling tended to be stronger in LQ ($\Delta^{66}Zn_{grain.filling}$: 0.32 to 0.62 ‰) compared to Oen ($\Delta^{66}Zn_{grain.filling}$: 0.14 to 0.47 ‰). The pattern of isotope fractionation during grain filling in the individual organs differed between the two soils: the smallest Zn isotope fractionation occurred for LQ in lower straw ($\Delta^{66}Zn_{grain.filling}$: 0.32 ‰ ± 0.05) and for Oen in flag leaves, rachis and spikelets ($\Delta^{66}Zn_{grain.filling}$: 0.14 to 0.20 ‰). The strongest enrichment of heavy Zn isotopes occurred for LQ in the peduncle ($\Delta^{66}Zn_{grain.filling}$: 0.62 ‰ ± 0.09), rachis ($\Delta^{66}Zn_{grain.filling}$: 0.52 ± 0.08 ‰) and spikelets ($\Delta^{66}Zn_{grain.filling}$: 0.49 ‰ ± 0.01) and for Oen in the peduncle ($\Delta^{66}Zn_{grain.filling}$: 0.47 ‰ ± 0.05).

We expected that the more of Zn was lost the stronger should be the apparent Zn isotope fractionation in the straw organs during grain filling. As suggested by Arnold et al. (2015), such a relation might be described by a Rayleigh model. However, the suggestion of Arnold et al. (2015) is based on the assumption of a closed system, implying no direct transfer from soil to grain during grain filling. In our study, the shoot has taken up additional Zn during grain filling (Table S4) which indicates that the relation between Zn mass and isotope fractionation followed also open system dynamics (Fry, 2006). We tried to establish a model for this relation by plotting the fractional change of Zn mass and the apparent isotope fractionation of each organ during grain filling (Fig. S3). However, no relationship appeared between these two variables.

Discussion

Isotope fractionation during vegetative growth

At flowering, spikelets were significantly enriched in light Zn isotopes (Fig. 3a). In general, it is proposed that Zn moves with the transpiration stream towards the transpiring, photosynthetically active organs such as expanded leaves (Fig. 4; Yamaji & Ma, 2014; Page & Feller, 2015). However, to also supply young plant organs that transpire less than fully developed leaves, Zn needs to be redistributed via the phloem (Yamaji & Ma, 2014; Page & Feller, 2015). Transport of Zn from expanded leaves to young leaves in wheat has been proven with a ^{65}Zn pulse label (Riesen & Feller, 2005; Page & Feller, 2005). After Zn was transported from the roots to the expanded leaves, it continuously moved to the youngest leaves during vegetative growth. In our study, the emerging ears were the youngest tissues of the wheat and accounted for 20 % of the total biomass at flowering (Table 1). Therefore, the majority of the transpiration stream must have been directed towards the organs of the lower shoot which accounted for 80 % of the total shoot biomass and only a minor part was directed to the ears until flowering. Among the organs of the ears, the spikelets contributed most to the DW of the ears at flowering (Table 1). Moreover, the spikelets started to develop in the stem excluded from sunlight. In that phase, the spikelets must have been fed by the phloem. Consequently, it is likely that the spikelets were mainly supplied with Zn via the phloem during vegetative growth, which might have led to the strong enrichment of the light Zn isotopes (Fig. 4).

Part of the oldest leaves that were already senescent at flowering were depleted in Zn and enriched in heavy isotopes compared to the photosynthetically active plant organs (Fig. 3a). As suggested previously (Stomph *et al.*, 2014; Schippers *et al.*, 2015), older plant organs might have acted already as phloem sources before grain filling started. The Zn isotope ratios in the senescent leaves suggest

that light isotopes were preferentially remobilized from the phloem sources (Fig. 4).

Isotope fractionation during grain filling

During grain filling, the wheat shoots accumulated markedly more Zn and became enriched in heavy Zn isotopes relative to the vegetative growth period (Fig. 2). Previous studies suggested that an enhanced Zn uptake into the shoot through enhanced transpiration (Couder *et al.*, 2015) or a stronger expression of low affinity transporters (John *et al.*, 2007) could have led to an enrichment of light isotopes in the shoot. Furthermore, an enrichment of heavy Zn isotopes in shoots of different plant species was ascribed to the release of organic compounds that mobilize additional Zn from the soil (Arnold *et al.*, 2010; Smolders *et al.*, 2013; Houben *et al.*, 2014). Therefore, the enrichment of heavy isotopes in the wheat shoots during grain filling might have been caused by an enhanced exudation of organic compounds such as phytosiderophores (Awad & Römheld, 2000; Puschenreiter *et al.*, 2017). Additionally, a previous study had shown that a significant fraction of a Zn radiolabel that was applied to young wheat shoots was recovered in the roots (Riesen & Feller, 2005). Thus, it cannot be excluded that Zn transport from shoot to root also occurred during grain filling in our experiment. Such a transport would occur via the phloem and would be expected to preferentially export the light Zn isotopes from the shoot to the roots (Fig. 4, see next sections for details).

The straw lost Zn and became enriched in heavy Zn isotopes during grain filling (Fig. 1, Fig. 3b). This observation is consistent with the enrichment of heavy Zn isotopes in senescent leaves at flowering that we ascribed to the remobilization of Zn from dying biomass to plant organs with a high Zn demand. At full maturity, about three quarters of the Zn was stored in the grains which were enriched in the light Zn isotopes relative to the straw. This observation is in agreement with the enrichment of the light Zn isotopes in grains of rice (Arnold *et al.*, 2015) or in the stems of reed, in which most of the Zn accumulated in the stem (Aucour *et al.*, 2015). Reproductive organs such as

grains demand high amounts of nutrients and exhibit low transpiration rates. Thus, grains are expected to be supplied with Zn via the phloem (Fig. 4, Herren & Feller, 1994; Pearson et al., 1995). Consequently phloem sources (straw) became enriched in heavy Zn isotopes, while phloem sinks (grain) became enriched in the light Zn isotopes during grain filling (Fig. 4). However, it is not clear if Zn that was additionally taken up from the soil during grain filling and loaded directly into the grain (without intermediate storage in stems or leaves, Page & Feller, 2015; Yoneyama et al., 2015) also contributed to the enrichment of light isotope in the grain.

The distinct Zn supply of the soils might have influenced the apparent Zn isotope fractionation during grain filling (Fig. 3b). Senescence of vegetative organs is an active process that is synchronized with nutrient demands in plants (Pottier *et al.*, 2014). Additionally, the timing and strength of phloem source and sink formation is influenced by stresses including inadequate micronutrient supply (Waters *et al.*, 2009; Schippers *et al.*, 2015). In this study, the low Zn supply of the Oen soil probably accelerated leaf senescence compared to LQ (Table 1). This suggests that reallocation of Zn within the shoot was already advanced at the flowering stage in Oen. Hence, the distinct timing of the Zn redistribution in the plant between the two study soils might have led to a weaker apparent isotope fractionation between straw and grain in Oen compared to LQ during grain filling (Fig. 3b). Furthermore, the distinct timing of phloem source and sink formation between the study soils also affect the Zn distribution on a plant organ level (Stomph *et al.*, 2014).

Although all organs became significantly enriched in heavy isotopes, the extent of isotope fractionation could not be related with the change of Zn mass during grain filling (Figure S3). In this period, the wheat took up additional Zn, thus, the isotope fractionation in the organs were not only determined by exporting light isotopes to the grains, but also by importing additional Zn. For each organ, the net change of mass during grain filling is known but the ratio of Zn that was in- and exported into an organ is unknown. The lack of a relation between the

change of Zn mass and apparent Zn isotope fractionation suggests that this ratio differed for each organ.

Biochemical processes causing Zn isotope fractionation in wheat

Our results demonstrated that phloem sources are enriched in heavy isotopes and phloem sinks in light isotopes at flowering and full maturity. Absorption to cell walls in the apoplastic space could be a key process in retaining heavy isotopes in the phloem sources such as the senescent straw organs (Fig. 4). Previous studies have shown that isotopically heavy Zn is preferentially sorbed to cell walls of diatoms (John *et al.*, 2007). In plant roots, heavy Zn was retained by O-ligands of pectin-containing cell walls, particularly the Zn fraction that is tightly bound to such cell walls (Aucour *et al.*, 2015; Tang *et al.*, 2016). In plant shoots, Zn has to cross apoplastic barriers during the transport from stems and leaves into the grain (Olsen & Palmgren, 2014). Each time Zn has to cross such an apolastic barrier, heavy Zn isotopes might be preferentially retained by sorption to cell walls. This explanation is strongly supported by speciation and isotope measurements of Zn in wetland plants that showed that more Zn was bound to cell walls in dead biomass that in living biomass while heavy isotopes were enriched in the dead biomass (Aucour et al. 2015, 2017). To further test this hypothesis, Zn should be extracted from leaf cell walls (Tukey, 1970) to measure Zn isotope ratios of cell wall bound Zn as recently demonstrated for roots (Tang *et al.*, 2016; Aucour *et al.*, 2017).

Complexation and compartmentalization of Zn in the cells might have also contributed to the enrichment of light isotopes in phloem sinks. Free Zn^{2+} concentrations in the cytosol is thought to be tightly controlled by complexation to organic ligands to prevent Zn toxicity (Blindauer & Schmid, 2010). Recently, nicotianamine (NA) has been suggested to play a major role as cytosolic Zn ligand (Clemens *et al.*, 2013). The O and N donor ligands of NA are expected to preferentially bind heavy Zn isotopes (Fujii *et al.*, 2014), which leads to an enrichment of light Zn isotopes in the cytosolic Zn^{2+} pool. The observed enrichment of light isotopes in young tissues and grains suggests that the light

Zn^{2+} pool in the cytosol is transported to these phloem sinks. In addition, excess Zn in the cytosol can also be stored in vacuoles where Zn is partly bound to organic acids (Aucour *et al.*, 2015) and released when the Zn demand is high (Olsen & Palmgren, 2014). Our data suggests that heavy isotopes are preferentially immobilized in the vacuoles likely through complexation of Zn to O donor ligands.

Besides vacuolar storage, Zn is also transported into other subcellular compartments such as the chloroplast and mitochondria where it is needed as a cofactor for enzymes (Nouet *et al.*, 2011). During senescence, the plant catabolizes these compartments in the straw, which might release Zn into the cytosol that was not available for retranslocation at the onset of grain filling (Pottier *et al.*, 2014). Our data suggest that the Zn pool released from these organelles must have been isotopically lighter than the retained Zn pool in the straw during grain filling.

In the alkaline phloem sap, Zn is chelated to remain soluble and transported towards phloem sinks (Alvarez-Fernandez *et al.*, 2014). For grasses, Zn has been found to be complexed dominantly by NA and Zn could have been entirely bound to NA (Nishiyama *et al.*, 2012). An incomplete complexation of Zn is expected to enrich the free Zn^{2+} pool in light isotopes (Fujii *et al.*, 2014). To date, it is not clear if Zn is unloaded into wheat grains from the phloem as dissociated light Zn^{2+} or as complexed Zn. The enrichment of light isotopes in the grains supports the assumption that light Zn^{2+} was transported into the grains. Isotope ratio measurements in phloem sap and grains could identify the final Zn species loaded into the grain.

Caldelas & Weiss (2017) showed in their review that diffusion and membrane transport favor light isotopes. Lighter Zn isotopes diffuse faster than heavy isotopes (Rodushkin *et al.*, 2004). Thus, diffusion-driven short-distance transport in e.g., the cell symplast could have led to an enrichment of light isotopes in phloem sinks. John et al. (2007) reported a preferential uptake of light Zn isotope into diatoms. They ascribed the isotope fractionation to the

membrane transport itself or to the diffusion from the nutrient solution towards the diatom. Therefore, it is likely that diffusion processes also contributed to the enrichment of light isotopes in the young tissues and grains. However, the chemically similar element Cd was enriched in heavy isotopes in wheat grains which was ascribed to complexation of light isotopes to thiols in root and straw (Wiggenhauser et al., 2016, details next section). This example suggests that isotope effects caused by complexation can superimpose isotope effects induced by diffusion and membrane transport as they also occur for Cd.

Inverse isotope fractionation of Zn and Cd in the same soil-plant systems

We compared Zn concentration and isotope ratios with previously published Cd data (Wiggenhauser et al., 2016) that were obtained in identical soil-plant systems (Table 2). In these systems, Zn concentrations were significantly higher but Cd concentrations were significantly lower in grains than in straw. Moreover, wheat grains were enriched with light Zn and heavy Cd isotopes while the straw was enriched with heavy Zn and light Cd isotopes. No significant isotope fractionation occurred between root and straw for Zn while Cd was enriched in light isotopes in the roots compared with the straw.

Cadmium and Zn behave chemically similar since they have a comparatively high electronegativity, easily form bonds with organic ligands and are not redoxsensitive (Kabata-Pendias, 2011; Maret & Moulis, 2013). However, compared with Zn, Cd is less stable in complexes with O and N but more stable in complexes with S ligands (Maret & Moulis, 2013). Additionally, Zn was about 200 to 330 times more abundant than Cd in the bulk soils of LQ and Oen (Table S1, S2). Because of the distinct chemical affinities and abundances of Cd and Zn, Cd might hardly compete with Zn for O and N ligands but it might effectively compete for S donor ligands in such systems (Maret & Moulis, 2013). Theoretical calculations revealed that lighter Zn isotopes bind to S ligands of organic compounds compared to O and N ligands (Fujii *et al.*, 2014). For Cd, calculations of isotope fractionation in hydrothermal fluids

revealed that lighter Cd bound to sulfides compared to e.g., hydroxides or nitrates (Yang *et al.*, 2015).

Our data suggest that the opposing isotope fractionation of Zn and Cd in wheat shoots was caused by the distinct affinities of these elements to functional groups of organic ligands. As previously suggested, isotopically light Cd might be dominantly bound to S ligands such as glutathione or phytochelatin immobilized in root and shoot cells (Wiggenhauser *et al.*, 2016). In contrast, isotopically heavy Zn is expected to be bound to O and N functional group of organic ligands such as phytosiderophores or NA (Fujii *et al.*, 2014; Marković *et al.*, 2017). Together, the opposing Zn and Cd isotope fractionation might provide information of the ligand environment of these elements that separates Zn from Cd in plants.

Acknowledgments

This study was funded by the Swiss Parliament via the National Research Program (NRP) 69 “Healthy Nutrition and Sustainable Food Production” (SNSF grant no. 406940_145195/1). We thank the farmers from the study sites for cooperation, Urs Feller for the discussion of the results and the technical staff of the Plant Nutrition Group at ETH Zurich for support in the growth chamber and in the laboratory.

Author Contribution

M.W. planned and conducted most of the experiment and laboratory work. M.I., E.F., W.W. M.B. strongly supported planning, data evaluation, data interpretation and manuscript writing. AK advised the soil and plant choice based on data from the Swiss Soil Monitoring Network. C.A. helped to adapt the column chemistry protocol, included the double-spike methodology and conducted the isotope measurements.

Literature

Alvarez-Fernandez A, Diaz-Benito P, Abadia A, Lopez-Millan A-F, Abadia J. **2014**. Metal species involved in long distance metal transport in plants. *Frontiers in Plant Science* **5**: 105.

Archer C, Andersen MB, Cloquet C, Conway TM, Dong S, Ellwood M, Moore R, Nelson J, Rehkämper M, Rouxel O, *et al.* 2017. Inter-calibration of a proposed new primary reference standard AA-ETH Zn for zinc isotopic analysis. *J. Anal. At. Spectrom.* **32**: 415–419.

Archer C, Vance D. **2004**. Mass discrimination correction in multiple-collector plasma source mass spectrometry: an example using Cu and Zn isotopes. *Journal of Analytical Atomic Spectrometry* **19**: 656.

Arnold T, Kirk GJD, Wissuwa M, Frei M, Zhao F, Mason TFD, Weiss DJ. **2010**. Evidence for the mechanisms of zinc uptake by rice using isotope fractionation. *Plant, cell & environment* **33**: 370–381.

Arnold T, Markovic T, Kirk GJD, Schönbächler M, Rehkämper M, Zhao FJ, Weiss DJ. **2015**. Iron and zinc isotope fractionation during uptake and translocation in rice (Oryza sativa) grown in oxic and anoxic soils. *Comptes Rendus - Geoscience* **347**: 397–404.

Aucour AM, Bedell JP, Queyron M, Magnin V, Testemale D, Sarret G. **2015**. Dynamics of Zn in an urban wetland soil-plant system: Coupling isotopic and EXAFS approaches. *Geochimica et Cosmochimica Acta* **160**: 55–69.

Aucour AM, Bedell JP, Queyron M, Tholé R, Lamboux A, Sarret G. **2017**. Zn Speciation and Stable Isotope Fractionation in a Contaminated Urban Wetland Soil– Typha latifolia System. *Environmental Science & Technology* **51**: 8350–8358.

Awad F, Römheld V. **2000**. Mobilization of heavy metals from contaminated calcareous soils by plant born, microbial and synthetic chelators and their uptake by wheat plants. *Journal of Plant Nutrition* **23**: 1847–1855.

Balter V, Lamboux A, Zazzo A, Télouk P, Leverrier Y, Marvel J, Moloney AP, Monahan FJ, Schmidt O, Albarède F. **2013**. Contrasting Cu, Fe, and Zn isotopic patterns in organs and body fluids of mice and sheep, with emphasis on cellular fractionation. *Metallomics* **5**: 1756–5901.

Bermin J, Vance D, Archer C, Statham PJ. **2006**. The determination of the isotopic composition of Cu and Zn in seawater. *Chemical Geology* **226**: 280–297.

Blindauer CA, Schmid R. **2010**. Cytosolic metal handling in plants: determinants for zinc specificity in metal transporters and metallothioneins. *Metallomics: integrated biometal science* **2**: 510–529.

Broadley M, Brown P, Cakmak I, Rengel Z, Zhao F. **2012**. Function of Nutrients. In: Marschner P, ed. Marschner's Mineral Nutrition of Higher Plants. London/Waltham, MA: Elsevier, 191–248.

Caldelas C, Dong S, Araus JL, Jakob Weiss D. **2011**. Zinc isotopic fractionation in Phragmites australis in response to toxic levels of zinc. *Journal of Experimental Botany* **62**: 2169–2178.

Caldelas C, Weiss DJ. **2017**. Zinc Homeostasis and isotopic fractionation in plants: a review. *Plant and Soil* **411**: 17–46.

Clemens S, Deinlein U, Ahmadi H, Höreth S, Uraguchi S. **2013**. Nicotianamine is a major player in plant Zn homeostasis. *BioMetals* **26**: 623–632.

Coleman JE. **1998**. Zinc enzymes. *Current opinion in chemical biology* **2**: 222–234.

Couder E, Mattielli N, Drouet T, Smolders E, Delvaux B, Iserentant A, Meeus C, Maerschalk C, Opfergelt S, Houben D. **2015**. Transpiration flow controls Zn transport in Brassica napus and Lolium multiflorum under toxic levels as evidenced from isotopic fractionation. *Comptes Rendus - Geoscience* **347**: 386–396.

Frossard E, Bucher M, Machler F, Mozafar A, Hurrell R. **2000**. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture* **80**: 861–879.

Fry B. **2006**. *Stable Isotope Ecology*. New York, NY: Springer New York.

Fujii T, Moynier F, Blichert-Toft J, Albarède F. **2014**. Density functional theory estimation of isotope fractionation of Fe, Ni, Cu, and Zn among species relevant to geochemical and biological environments. *Geochimica et Cosmochimica Acta* **140**: 553–576.

Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A, Groneberg DA. **2006**. The toxicity of cadmium and resulting hazards for human health. *Journal of Occupational Medicine and Toxicology* **1**: 1–6.

Herren T, Feller U. **1994**. Transfer of zinc from xylem to phloem in the peduncle of wheat. *Journal of Plant Nutrition* **17**: 1587–1598.

Houben D, Sonnet P, Tricot G, Mattielli N, Couder E, Opfergelt S. **2014**. Impact of root-induced mobilization of zinc on stable Zn isotope variation in the soil-plant system. *Environmental Science and Technology* **48**: 7866–7873.

John SG, Geis RW, Saito MA, Boyle EA. **2007**. Zinc isotope fractionation during high-affinity and low-affinity zinc transport by the marine diatom Thalassiosira oceanica. *Limnology and Oceanography* **52**: 2710–2714.

622 **Kabata-Pendias A**. **2011**. *Trace elements in Soil and Plants*. Boca Raton: CRC Press.

623 **Khan MA, Castro-Guerrero N, Mendoza-Cozatl DG**. **2014**. Moving toward a precise nutrition: preferential loading of seeds with
624 essential nutrients over non-essential toxic elements. *Frontiers in plant science* **5**: 51.

625 **Maret W, Moulis J-M**. **2013**. The Bioinorganic Chemistry of Cadmium in the Context of Its Toxicity. In: Sigel A, Sigel H, Siegel RKO,
626 eds. Cadmium: From Toxicity to Essentiality. Springer Netherlands, 1–29.

627 **Marković T, Manzoor S, Humphreys-Williams E, Kirk GJ, Vilar R, Weiss DJ**. **2017**. Experimental Determination of Zinc Isotope
628 Fractionation in Complexes with the Phytosiderophore 2′-Deoxymugeneic Acid (DMA) and Its Structural Analogues, and Implications
629 for Plant Uptake Mechanisms. *Environmental Science & Technology* **51**: 98–107.

630 **Mertens J, Smolders E**. **2013**. Zinc. In: Alloway BJ, ed. Heavy Metals in Soils - Trace metals and metalloids in soils and their
631 bioavailability. Dordrecht: Springer, 465–493.

632 **Moeller K, Schoenberg R, Pedersen R-B, Weiss D, Dong S**. **2012**. Calibration of the New Certified Reference Materials ERM-AE633
633 and ERM-AE647 for Copper and IRMM-3702 for Zinc Isotope Amount Ratio Determinations. *Geostandards and Geoanalytical*
634 *Research* **36**: 177–199.

635 **Moynier F, Pichat S, Pons ML, Fike D, Balter V, Albarède F**. **2009**. Isotopic fractionation and transport mechanisms of Zn in plants.
636 *Chemical Geology* **267**: 125–130.

637 **Nishiyama R, Kato M, Nagata S, Yanagisawa S, Yoneyama T**. **2012**. Identification of Zn–Nicotianamine and Fe–2′-Deoxymugineic
638 Acid in the Phloem Sap from Rice Plants (Oryza sativa L.). *Plant and Cell Physiology* **53**: 381–390.

639 **Nouet C, Motte P, Hanikenne M**. **2011**. Chloroplastic and mitochondrial metal homeostasis. *Trends in Plant Science* **16**: 395–404.

640 **Olsen LI, Palmgren MG**. **2014**. Many rivers to cross: the journey of zinc from soil to seed. *Frontiers in Plant Science* **5**: 30.

641 **Page V, Feller U**. **2005**. Selective transport of zinc, manganese, nickel, cobalt and cadmium in the root system and transfer to the leaves
642 in young wheat plants. *Annals of Botany* **96**: 425–434.

643 **Page V, Feller U**. **2015**. Heavy Metals in Crop Plants: Transport and Redistribution Processes on the Whole Plant Level. *Agronomy* **5**:
644 447–463.

645 **Palmgren MG, Clemens S, Williams LE, Krämer U, Borg S, Schjørring JK, Sanders D**. **2008**. Zinc biofortification of cereals:
646 problems and solutions. *Trends in Plant Science* **13**: 464–473.

647 **Pearson JN, Rengel Z, Jenner CF, Graham RD**. **1995**. Transport of zinc and manganese to developing wheat grains. *Physiologia*
648 *Plantarum* **95**: 449–455.

649 **Pinna K, Woodhouse LR, Sutherland B, Shames DM, King JC**. **2001**. Exchangeable zinc pool masses and turnover are maintained in
650 healthy men with low zinc intakes. *The Journal of nutrition* **131**: 2288–94.

651 **Ponnamperuma FN, Cayton MT, Lantin RS**. **1981**. Dilute hydrochloric acid as an extractant for available zinc, copper and boron in
652 rice soils. *Plant and Soil* **61**: 297–310.

653 **Pottier M, Masclaux-Daubresse C, Yoshimoto K, Thomine S**. **2014**. Autophagy as a possible mechanism for micronutrient
654 remobilization from leaves to seeds. *Frontiers in plant science* **5**: 11.

655 **Puschenreiter M, Gruber B, Wenzel WW, Schindlegger Y, Hann S, Spangl B, Schenkeveld WDC, Kraemer SM, Oburger E**. **2017**.
656 Phytosiderophore-induced mobilization and uptake of Cd, Cu, Fe, Ni, Pb and Zn by wheat plants grown on metal-enriched soils.
657 *Environmental and Experimental Botany* **138**: 67–76.

658 **Riesen O, Feller U**. **2005**. Redistribution of Nickel, Cobalt, Manganese, Zinc, and Cadmium via the Phloem in Young and Maturing
659 Wheat. *Journal of Plant Nutrition* **28**: 421–430.

660 **Rodushkin I, Stenberg A, Andrén H, Malinovsky D, Baxter DC**. **2004**. Isotopic Fractionation during Diffusion of Transition Metal
661 Ions in Solution. *Analytical Chemistry* **76**: 2148–2151.

662 **Schippers JHM, Schmidt R, Wagstaff C, Jing HC**. **2015**. Living to Die and Dying to Live: The Survival Strategy behind Leaf
663 Senescence. *Plant Physiology* **169**: 914–930.

664 **Smolders E, Versieren L, Shuofei D, Mattielli N, Weiss D, Petrov I, Degryse F**. **2013**. Isotopic fractionation of Zn in tomato plants
665 suggests the role of root exudates on Zn uptake. *Plant and Soil* **370**: 605–613.

666 **Stomph TJ, Jiang W, Van Der Putten PEL, Struik PC**. **2014**. Zinc allocation and re-allocation in rice. *Frontiers in Plant Science* **5**: 8.

667 **Tang Y, Cloquet C, Deng T, Sterckeman T, Echevarria G, Yang W, Morel J, Qiu R**. **2016**. Zinc Isotope Fractionation in the
668 Hyperaccumulator Noccaea caerulescens and the Nonaccumulating Plant Thlaspi arvense at Low and High Zn Supply. *Environmental*
669 *Science & Technology* **50**: 8020–8027.

670 **Tukey HB**. **1970**. The Leaching of Substances from Plants. *Annual Review of Plant Physiology* **21**: 305–324.

671 **Viers J, Oliva P, Nonell A, Gélabert A, Sonke JE, Freydier R, Gainville R, Dupré B**. **2007**. Evidence of Zn isotopic fractionation in a
672 soil-plant system of a pristine tropical watershed (Nsimi, Cameroon). *Chemical Geology* **239**: 124–137.

673 **Waters BM, Uauy C, Dubcovsky J, Grusak MA**. **2009**. Wheat (Triticum aestivum) NAM proteins regulate the translocation of iron,
674 zinc, and nitrogen compounds from vegetative tissues to grain. *Journal of Experimental Botany* **60**: 4263–4274.

675 **Wiggenhauser M, Bigalke M, Imseng M, Müller M, Keller A, Murphy K, Kreissig K, Rehkämper M, Wilcke W, Frossard E**.
676 **2016**. Cadmium isotope fractionation in soil–wheat systems. *Environmental Science & Technology* **50**: 9223–9231.

677 **Yamaji N, Ma JF**. **2014**. The node, a hub for mineral nutrient distribution in graminaceous plants. *Trends in Plant Science* **19**: 556–563.

678 **Yang J, Li Y, Liu S, Tian H, Chen C, Liu J, Shi Y**. **2015**. Theoretical calculations of Cd isotope fractionation in hydrothermal fluids.
679 *Chemical Geology* **391**: 74–82.

680 **Yoneyama T, Ishikawa S, Fujimaki S**. **2015**. Route and Regulation of Zinc, Cadmium, and Iron Transport in Rice Plants (Oryza sativa
681 L.) during Vegetative Growth and Grain Filling: Metal Transporters, Metal Speciation, Grain Cd Reduction and Zn and Fe
682 Biofortification. *International Journal of Molecular Sciences* **16**: 19111–19129.

683 **Zhao Y, Vance D, Abouchami W, de Baar HJW**. **2014**. Biogeochemical cycling of zinc and its isotopes in the Southern Ocean.
684 *Geochimica et Cosmochimica Acta* **125**: 653–672.

685

Supporting Information

Table 1 Dry weights (DW) and Zn concentrations in wheat shoots (*Triticum aestivum* L.).

	Dry Weight				Zn concentration			
	LQ[d]		Oen[d]		LQ[d]		Oen[d]	
	flowering	full maturity	flowering	full maturity	flowering	full maturity	flowering	full maturity
	mean se[a]	mean se[a]	mean se[a]	mean se[a]	mean se[a]	mean se[a]	mean se[a]	mean se[a]
	g	g	g	g	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
shoot[b]	5.61 ± 0.25 C	17.8 ± 0.60 A	6.42 ± 0.91 B	12.9 ± 0.38 A	28.5 ± 1.16 A	23.7 ± 0.63 B	15.8 ± 1.07 A	15.6 ± 0.60 A
straw[b]		8.17 ± 0.44 B		6.85 ± 0.25 B		12.3 ± 0.24 C		6.7 ± 0.30 B
grains[b]		9.59 ± 0.17 a		6.03 ± 0.14 a		33.3 ± 0.93 a		25.8 ± 1.06 a
lower straw[b]	3.96 ± 0.25 a	3.70 ± 0.28 b	3.97 ± 0.74 a	3.96 ± 0.17 b	29.5 ± 1.45 a	7.65 ± 0.60 c	15.2 ± 3.11 b	4.95 ± 0.15 de
flag leaf[c]	0.32 ± 0.02 c	0.56 ± 0.07 c	0.25 ± 0.02 c	0.30 ± 0.02 de	26.9 ± 0.57 ab	5.93 ± 0.16 c	17.2 ± 1.62 ab	4.65 ± 0.13 e
peduncle[c]	0.12 ± 0.01 d	0.44 ± 0.04 c	0.12 ± 0.02 d	0.22 ± 0.04 e	20.2 ± 1.00 c	15.7 ± 0.57 b	15.2 ± 0.94 b	7.46 ± 1.85 d
rachis[c]	0.13 ± 0.02 d	0.53 ± 0.02 c	0.18 ± 0.01 cd	0.42 ± 0.05 d	18.0 ± 1.33 c	18.0 ± 2.00 b	17.3 ± 1.57 ab	14.6 ± 1.85 b
spikelets[c]	0.84 ± 0.06 b	2.94 ± 0.13 b	1.15 ± 0.11 b	1.95 ± 0.05 c	29.3 ± 0.86 a	17.9 ± 0.92 b	22.3 ± 0.52 a	8.85 ± 0.65 c
lower leaves senescent[c]	0.25 ± 0.06 c		0.74 ± 0.03 b		21.5 ± 2.17 bc		7.4 ± 0.07 c	

[a]se denotes standard error of n = 4 samples. Capital letters denote statistical difference (p < 0.05) between shoot at flowering and full maturity and straw at full maturity. Small letters denote statistical differences (p < 0.05) within each column.
[b]Number of tiller: flowering LQ 12 ± 0.41, Oen 10 ± 0.63; full maturity LQ 11 ± 0.91, Oen 6 ± 0.00
[c]At flowering, numbers represent DM and Zn concentration of tillers 1-3, all other tillers were defined as lower straw. At full maturity, numbers represent DW and Zn concentration of all tilllers.
[d]Origin of the soils (LQ = Landquart, Oen = Oensingen)

Table 2 Comparison of Zn and Cd isotope fractionation in identical soil-wheat systems.

	LQ[c]				Oen[c]			
	Zn conc.		Cd conc.[b]		Zn conc.		Cd conc.[b]	
	mean	se	mean	se	mean	se	mean	se
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	
grain	33.3	± 0.93 a	0.03	± 0.01 b	25.8	± 1.06 a	0.06	± 0.01 b
straw	12.3	± 0.24 b	0.10	± 0.03 b	6.69	± 0.30 c	0.14	± 0.03 b
roots	28.5	± 3.13 a	0.30	± 0.04 a	14.0	± 0.67 b	0.35	± 0.02 a
	δ^{66}Zn		δ^{114}Cd[b]		δ^{66}Zn		δ^{114}Cd[b]	
	mean	se[a]	mean	se[a]	mean	se[a]	mean	se[a]
	‰		‰		‰		‰	
grain	0.18	± 0.00 b	0.66	± 0.04 a	0.45	± 0.01 b	0.62	± 0.10 a
straw	0.49	± 0.03 a	0.37	± 0.04 b	0.66	± 0.03 a	0.52	± 0.07 b
roots	0.63	± 0.05 a	0.13	± 0.03 c	0.56	± 0.10 ab	0.11	± 0.05 c

[a]se denotes standard error of n = 3 samples and small and capital letters denote significant differences of the mean values for Zn and Cd, respectively (p < 0.05).
[b]Cadmium data were taken from Wiggenhauser et al. (2016); δ^{114}Cd denotes isotope ratio of ^{114}Cd/^{110}Cd expressed relative to a Cd isotope standard (NIST 3108).
[d]Origin of the soils (LQ = Landquart, Oen = Oensingen)

700 **Legends**

Fig. 1: Change of Zn mass per tiller during grain filling in wheat (*Triticum aestivum* L.). LQ (Landquart, red) and Oen (Oensingen, blue) denote the origins of the arable soils used for the pot experiment. The Zn mass balance in straw organs was calculated as difference between mean Zn mass at full maturity and at flowering (n = 4). Error bars illustrate ± se of calculated standard errors. Letters denote significant differences among shoot organs in LQ and Oen.

Fig. 2: Zinc isotope ratios at flowering and full maturity (circles, red: soil from Landquart, triangles, blue: soil from Oensingen) in wheat shoots (*Triticum aestivum* L.). Error bars illustrate ± se of n = 3 replicates. Italic and regular letters denote significant differences between the shoots for LQ and Oen, respectively.

Fig. 3: a) Zn isotope ratios in shoot organs at flowering (green, circles) and full maturity (brown, triangles) in wheat (*Triticum aestivum* L.). LQ (Landquart) and Oen (Oensingen) denote the origins of the arable soils used for the pot experiment. a) Error bars illustrate ± se of n = 3 replicates. Italic and regular letters denote significant differences among shoot organs at flowering and full maturity, respectively. b) Apparent Zn isotope fractionation during grain filling in straw organs calculated as difference between mean Zn isotope ratios at full maturity and at flowering (n = 3). Error bars illustrate ± se of calculated standard errors. Letters denote significant differences among shoot organs in LQ and Oen.

Fig. 4: Conceptual model summarizing major pathways and Zn isotope fractionation in wheat shoots. 1) Zn moves with the transpiration stream towards expanded leaves such as the lower shoot and needs to be unloaded from the xylem to enter the symplast. 2) During vegetative growth, expanded leaves might preferentially release light Zn isotopes through the phloem towards developing tissues such as the spikelets. The released light Zn isotopes are partly replaced by Zn derived from the xylem as long as the leaves are transpiring. 3) In senescent tissues, Zn is remobilized towards phloem sinks and is not replaced anymore which leads to a stronger enrichment of heavy Zn isotopes. 4) The grains are a strong phloem sink and are enriched in light Zn isotopes relative to the rest of the shoot because of preferential accumulation of light Zn isotopes from remobilized light Zn in straw during maturation. 5) Direct transport from soil to grain by xylem to phloem transfer in stem tissues (unknown contribution to isotope fractionation). 6) During grain filling, the Zn transferred from root to shoot becomes isotopically heavier because of the acquisition of heavy Zn isotopes caused by root exudation of organic acids and/or 7) because of the export of light Zn isotopes via the phloem from shoot to root. Figure layout based on Blindauer & Schmid (2010).

701

702

703

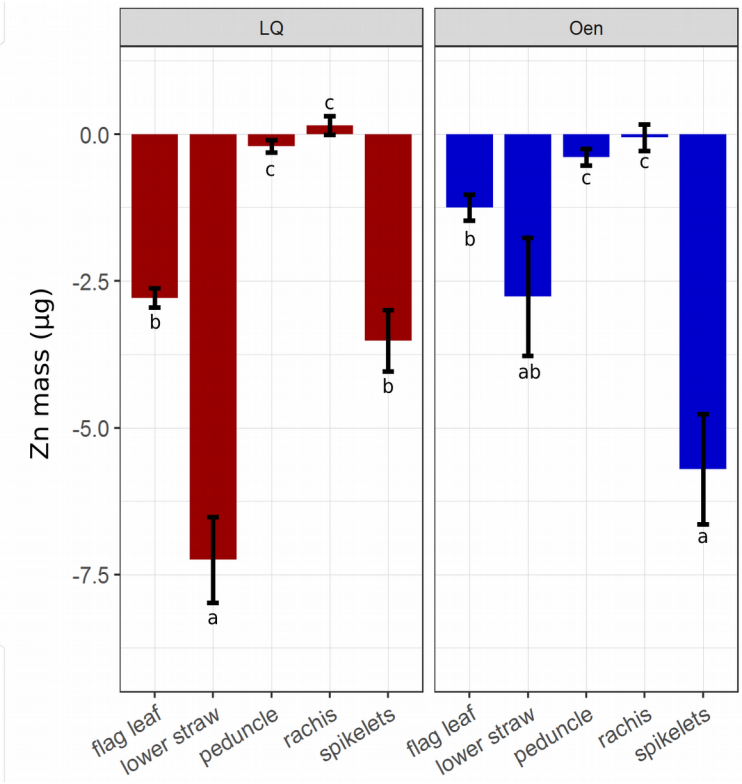

Figure 1

705

706

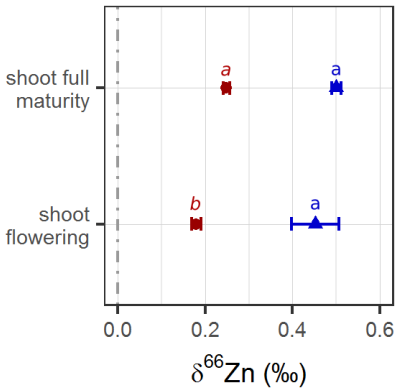

Figure 2

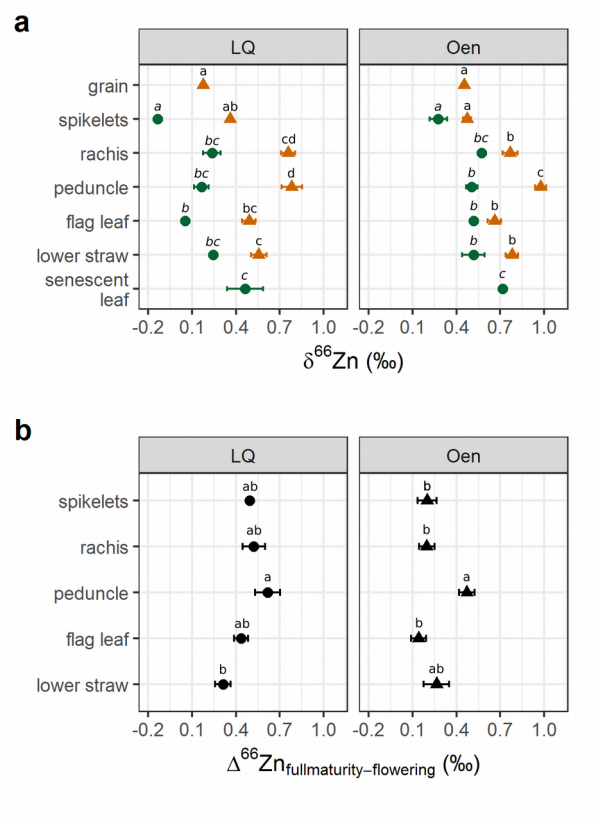
a
LQ
Oen
grain
spikelets
rachis
peduncle
flag leaf
lower straw
senescent leaf
-0.2 0.1 0.4 0.7 1.0
δ66Zn (‰)
b
LQ
Oen
spikelets
rachis
peduncle
flag leaf
lower straw
-0.2 0.1 0.4 0.7 1.0
Δ66Znfullmaturity−flowering (‰)

Figure 3

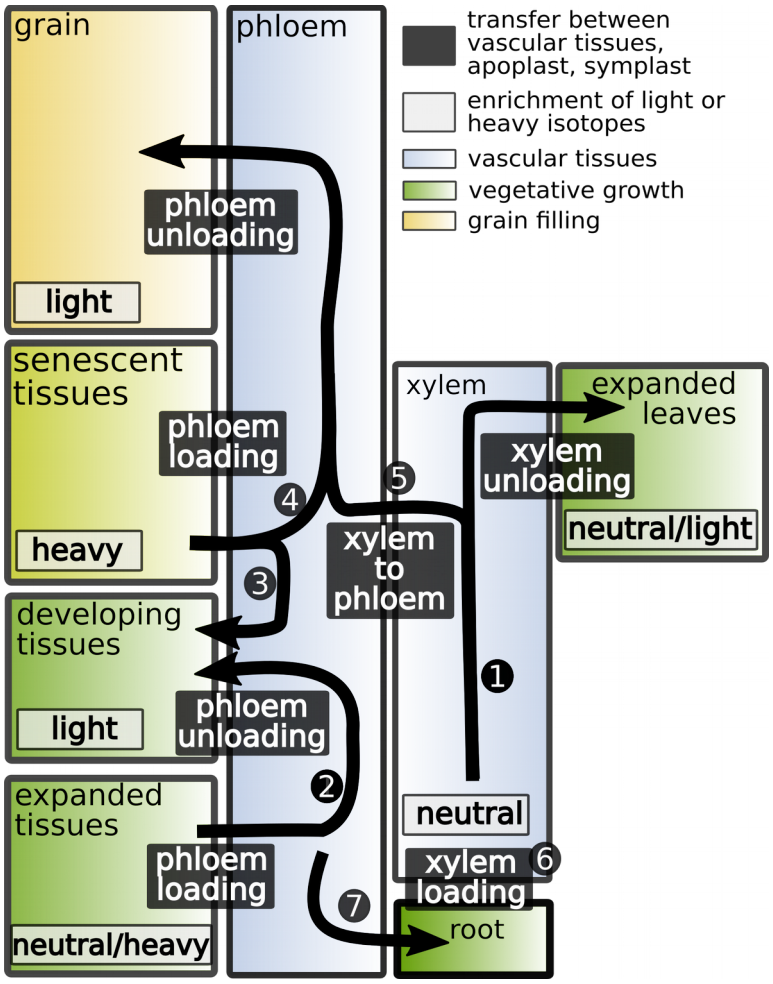
grain
phloem
transfer between vascular tissues, apoplast, symplast
enrichment of light or heavy isotopes
vascular tissues
vegetative growth
grain filling
phloem unloading
light
senescent tissues
phloem loading
heavy
xylem
expanded leaves
xylem unloading
neutral/light
5
4
xylem to phloem
3
developing tissues
light
phloem unloading
1
2
expanded tissues
phloem loading
neutral/heavy
neutral
xylem loading
6
7
root

Figure 4